J. Zool., Lond. (2000) **252**, 293–302

Net evolutionary trajectories of body shape evolution within a microgeographic radiation of threespine sticklebacks (*Gasterosteus aculeatus*)

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(Accepted 21 October 1999)

Abstract

Following deglaciation of the Cook Inlet region of Alaska approximately 16 000 years ago, anadromous threespine stickleback (*Gasterosteus aculeatus*) rapidly colonized emerging lakes and rivers forming resident, freshwater populations. Although the precise body shape of the ancestral marine population is unknown, marine sticklebacks sampled from both Pacific and Atlantic sites present remarkably little body shape variation among populations, which suggests that the morphology of any of the marine populations could be used to represent the ancestral phenotype. To infer the net evolutionary trajectories of body shape change in the Cook Inlet radiation, derived body shapes of lacustrine samples were compared to the presumptive, primitive body shape, represented by the mean shape of two anadromous samples from Cook Inlet. In general, some derived body shape traits are shared by all freshwater populations but many traits evolved in opposite directions. The principal axes of shape variation among freshwater sample means were computed using Principal Components Analysis. The strong correlation between the direction of the principal component axes and lake habitat variables suggest that populations evolved toward selection peaks that are biased along the component axes due to biotic and abiotic features of the lakes.

Key words: ancestor, body shape, geometric morphometrics, adaptive radiation, *Gasterosteus aculeatus*

INTRODUCTION

Threespine stickleback, *Gasterosteus aculeatus*, occur in thousands of lakes around Cook Inlet, Alaska and exhibit considerable phenotypic diversity. Major components of interpopulation variation include body shape (Walker, 1996, 1997), trophic morphology (Francis *et al*., 1986; Caldecutt & Adams, 1998), armour morphology (Bell *et al*., 1993; Bell & Ortí, 1994; Borgeois *et al*., 1994), life history (Baker, 1994; Baker *et al*., 1998) and reproductive behaviour (Foster, 1995). At the onset of the present interglacial approximately 16 000 years ago (Reger & Pinney, 1995), emerging streams and lakes in the Cook Inlet region were rapidly colonized by threespine stickleback (hereafter, stickleback). Freshwater refugia were limited or absent in the Cook Inlet region during the last glaciation (Reger & Pinney, 1995) and anadromous populations are the most likely source of the colonizing individuals (Bell *et al*., 1993; Bell & Foster, 1994). Anadromous stickleback are known both to rapidly colonize recently defaunated lakes and to adapt to freshwater environments at extremely high rates (Klepaker, 1993; M. A. Bell, pers. obs.).

In contrast to the microgeographic radiations of freshwater sticklebacks, such as that from Cook Inlet, anadromous populations sampled throughout the Pacific basin present considerably less phenotypic variation. The minor geographic variation among anadromous populations suggests a static evolutionary history of the anadromous phenotype for at least several millions of years (Bell & Foster, 1994). This hypothesis is supported by the similarity of Miocene marine stickleback from eastern Russia and California to modern anadromous stickleback (Bell, 1994). Nevertheless, the repeated and rapid evolution of derived freshwater phenotypes throughout its range suggests a dynamic evolutionary history of the stickleback as a whole. Williams (1992) coined the phrase ‘phylogenetic raceme’ to describe this unusual history. In this concept, a temporally stable mainstem lineage gives rise to numerous, phenotypically diverse, phylogenetically independent and largely ephemeral, derived lineages (Bell & Foster, 1994).

The unusual history of freshwater sticklebacks inhabiting recently deglaciated regions has interesting

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implications for analysing patterns of phenotypic evolution within the radiation. For example, ancestral character states for the derived freshwater populations should be those states present in extant anadromous samples. Thus, the polarity of character state evolution can be inferred by directly comparing freshwater with marine populations (Bell & Foster, 1994). This conclusion rests on the assumption that body shape in marine sticklebacks has experienced only trivial temporal and geographic evolution. In this study, we explored the hypothesis of geographic and temporal stability of body shape among anadromous stickleback sampled throughout much of its range and, finding little variation, conclude that the marine form can be considered the ancestral phenotype. We then compared body shape between a consensus anadromous and 40 freshwater samples from Cook Inlet in order to describe replicated patterns of historical transformation in stickleback body shape.

METHODS

Samples

A previous paper (Walker, 1997) described shape variation among 40 lacustrine populations of stickleback sampled from 2 regions that drain into Cook Inlet, Alaska: the Matanuska-Susitna (Mat-Su) Valley; and the Kenai Peninsula. The sample sizes and locations of each site are given in Walker (1997) and Bell & Ortí (1994), respectively. The populations were sampled to contrast 2 lake variables: relative littoral area; and presence or absence of native predatory fishes (Walker, 1997).

In addition to the 40 freshwater (FW) samples, 2 anadromous samples from Cook Inlet were chosen to represent the ancestral phenotype. The anadromous populations were sampled from 2 small, slow moving streams that provide suitable spawning habitat for anadromous stickleback. An anadromous life history was assumed in these samples based on the absence of individuals from these streams outside of the breeding season. These 2 streams are Rabbit Slough, in the Mat-Su Valley (149°15′51″W. Longitude; 61°32′26″N. Latitude), and an unnamed slough just north of the mouth of the Kenai River (151°16′25″W. Longitude; 60°33′15″N. Latitude) within the Kenai Peninsula.

Because the body shape of the anadromous stickleback that currently spawn in the Cook Inlet region might not be representative of the anadromous stickleback that colonized the fresh waters of the region following deglaciation, we also compared the Cook Inlet anadromous stickleback with marine stickleback sampled throughout much of the stickleback's range. These global marine samples were from Stockton Springs, Maine, USA ($n = 18$), Netart's Bay, Oregon, USA ($n = 42$), Puck Bay, Poland ($n = 10$), and the White Sea ($n = 10$). Because we do not know if these populations spawn in estuaries or freshwater streams, we use *marine* to refer to any population that overwinters in the sea.

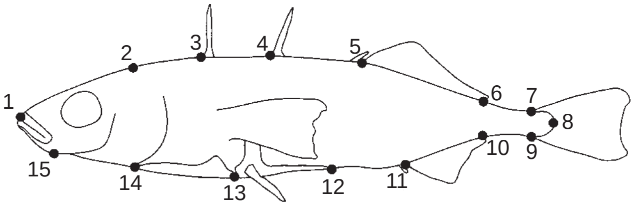

Fig. 1. Locations of the 15 landmarks digitized and analysed in this study. See Walker (1997) for a more detailed description of these landmarks.

Morphometric and statistical analysis

The locations of the 15 landmarks measured on each specimen are illustrated in Fig. 1 and described in detail in Walker (1997). The configuration of p landmarks for a specimen is referred to as a figure (Goodall, 1991). All figures were rotated to align the first and eighth landmark (i.e. the chord for standard length) along the horizon and superimposed with a generalized orthogonal least squares superimposition (Rolf & Slice, 1990) using the software Morphometrika (Walker, 1997). The mean figure (group mean figure) from each of the 46 sites was computed from this superimposition.

The grand mean figure (mean of the group mean figures) was used to compute principal warps, which are simply the eigenvectors of a $p \times p$ matrix (the bending energy matrix) whose elements are inversely related to the distance among the p landmarks of the reference figure (Bookstein, 1991). The first p-3 eigenvectors estimate a subspace without affine (uniform deformation across the entire figure) variation. The superimposed group mean figures from both FW and marine sites were projected into this subspace, effectively using the minimal bending energy criterion to remove all translation, rotation, scaling, and shearing variation from the data. These $2 \times (p\text{-}3)$ scores are the partial warp scores (Rohlf, 1993). For each figure, a pair of uniform scores, u_1 and u_2, were computed following Bookstein (1996). Although many variations of uniform scores exist, the scores developed by Bookstein (1996) are in the same scale as the partial warp scores and allow the full affine shape and non-affine shape variation to be analysed together. The combined partial warp and uniform (PW-U) scores were the data for all subsequent analyses.

We used Principal Components Analysis (PCA) to analyse variation patterns. Many different protocols for analysing geometric morphometric variation using PCA have been suggested. Tabachnick & Bookstein (1990) analysed principal components of shape coordinates, which are the coordinates of the figures following a 2-point registration (TPR). Inferences drawn from the direction of the components of shape variation are necessarily ambiguous, however, because results must be interpreted with respect to an invariant baseline (Walker, 1993*b*). To reduce the effects of misplaced variation among the landmarks, principal components can be computed from the residual variation following alignment of the figures by least squares or resistant fit superimpositions (Walker, 1993*a*, 1996). Bookstein (1991) developed a PCA, Relative Warp Analysis (RWA), based on the decomposition of the thin-plate spline into a series of deformations at different spatial scales (partial warps). In its original formulation (Bookstein, 1991), the RWA weighted the variation by the inverse of its associated scale (hence, relative warps are components of variation relative to the magnitude of spatial scale). Rohlf (1993) generalized the RWA, allowing a variety of weightings, but advocated weighting all scales of variation equally (as in traditional PCA). Until recently (Auffray *et al.*, 1996; Rohlf, Loy & Corti, 1996), all RWAs computed the components of only the non-affine variation, which was contained in the partial warp scores. A PCA of the non-affine shape variation can be misleading since there will usually be residual shape variation (the uniform portion) correlated with the components (Walker, 1996). Rohlf *et al.* (1996) suggested several alternatives for including the affine shape variation in an analysis: (1) the addition of the uniform scores, u_1 and u_2, to the set of partial warp scores; (2) orthogonal projection of the co-ordinates of the superimposed landmarks into the space defined by the full set of eigenvectors of the bending energy matrix; (3) the full set of $2 \times p$ superimposed co-ordinates. PCAs of (2) and (3) are identical, regardless of the initial superimposition algorithm, since PCA is invariant to orthogonal rotations of the original data. If the figures are initially fit by the Generalized Orthogonal Least Squares algorithm of Rohlf & Slice (1990), all 3 methods are identical. We advocated the direct analysis of the superimposed co-ordinates (Walker, 1993*a*,*b*, 1996, 1997) prior to the development of the uniform scores of Bookstein (1996). For this analysis, we use the PW-U scores to compute the principal components.

As an initial exploration of variation among FW sites and among marine sites, and differences between FW and marine sites, we ordinated all 46 group means using PCA ($\mathrm{PCA_{TOT}}$). Eigenvectors were computed from the covariance matrix of PW-U scores.

Shape differences between the 2 Cook Inlet anadromous sticklebacks proved to be small (see Results) relative to differences among the FW forms, and we used the average of the PW-U scores of the 2 Cook Inlet anadromous samples to represent the ancestral shape. We refer to these averaged scores as the Cook Inlet Marine Form. The vector of differences, z_i, between the Cook Inlet Marine Form PW-U scores and the scores from the ith FW sample approximates the direction of net differentiation (in principal warp space) from the marine ancestor to the derived FW form. We refer to the z_i as the *difference vectors*.

We normalized the 40 difference vectors and computed their mean, $\bar{z}$. In order to explore the effects of the choice of marine form on each z_i, we computed normalized difference vectors taken with respect to the PW-U scores from each of the global marine samples individually. We then used a vector correlation to compare $\bar{z}$ to the mean difference vectors with respect to the individual marine samples.

One goal of this analysis was to describe common patterns of net divergence. A PCA of the difference vectors is equivalent to a PCA among freshwater sample means ($\mathrm{PCA_{FW}}$), since the PW-U scores and the difference vectors differ by a constant vector and, therefore, the covariance matrices are equivalent. We explored an alternative method by using an eigenvalue decomposition of the sums of squares and cross-products (SSCP) matrix of the PW-U scores centered around the values of the Cook Inlet Marine Form (and thus the uncentered SSCP matrix of the difference vectors). This proved undesirable because the correlation between the first and later components made the interpretation difficult (all of the components after the first were uncorrelated, as in conventional PCA). Using the eigenvectors of the FW PW-U scores, we projected the Cook Inlet Marine Form into the $\mathrm{PCA_{FW}}$ space in order to polarize the direction of differentiation from the ancestral to the derived phenotypes.

To facilitate interpretation of the patterns of shape variation, we transformed all eigenvector coefficients, which are relative to the PW-U space, back into the space of the landmarks and scaled them as correlations between the jth principal component (PC) and the x or y co-ordinates of the ith landmark,

$$1_{ij} = \mathrm{cor}\,(\boldsymbol{y}_\mathrm{i}, \boldsymbol{s}_\mathrm{j}) \qquad (1)$$

where 1_{ij} can be interpreted as 'loadings', y_i is the vector of superimposed x or y co-ordinates for the ith landmark, and $\boldsymbol{s_j}$ is the vector of the jth PC scores.

The pattern of loadings on a principal component axis and its associated pattern of shape variation can be visualized using a thin-plate spline. We scaled the coefficients as root covariances between the principal components and the superimposed landmarks,

$$v_{ij} = \sqrt{\mathrm{cov}\,(\boldsymbol{y}_i, \boldsymbol{s}_j)} \qquad (2)$$

The pair of coefficients at landmark i describes the resultant direction of differentiation associated with principal component j (Walker, 1993*b*, 1996, 1997). The figure constructed from the locations of the tips of each of these resultant vectors describes a hypothetical extreme at one end of the jth PC axis. An extreme hypothetical shape at the opposite end of the jth PC axis can be constructed from the tips of the resultant vectors multiplied by -1 (reversing the direction of the vectors). We used the thin-plate spline deformation from the grand mean figure to the hypothetical extreme figures to describe the patterns of shape differentiation along each PC axis.

RESULTS

The ordination of the different samples ($\mathrm{PCA_{TOT}}$) indicates that (1) globally sampled marine sticklebacks present markedly less shape variation than do Cook Inlet FW sticklebacks and (2) the region of the shape

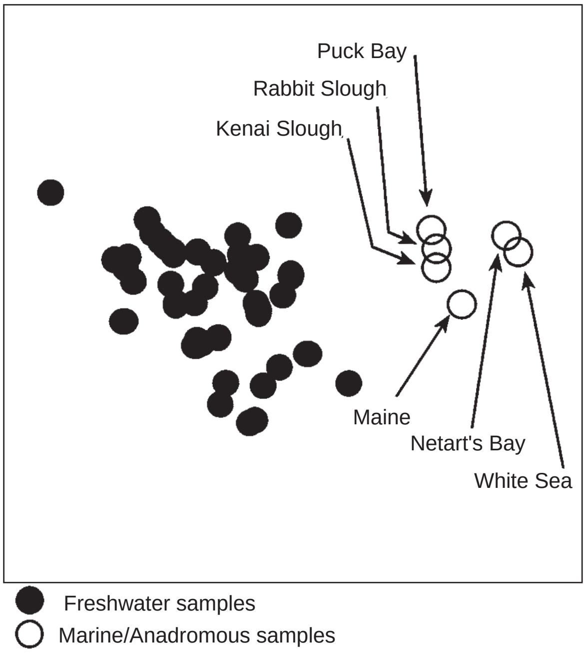

Fig. 2. Ordination of the 40 freshwater group-mean-figures and six global-marine group-mean-figures in the principal component space computed using both freshwater and marine data ($\mathrm{PCA_{TOT}}$). The ordination shows that (1) marine sticklebacks sampled on a global scale present less shape variation than freshwater sticklebacks sampled on a microgeographic scale (the Cook Inlet region of Alaska), (2) body shape in marine sticklebacks is outside the variation in body shape among freshwater sticklebacks, and (3) any of the marine samples would result in a reasonable proxy for the ancestral body shape.

space occupied by the marine samples lies distinctly outside the region occupied by the FW samples (Fig. 2).

Patterns of body shape variation among FW sticklebacks are illustrated in Fig. 3. For each component, the two hypothetical shapes and associated deformations represent opposite extremes along the axis' continuum (the magnitude of the extremes is twice the actual magnitude of variation).

The first FW principal component (PC1) contrasts fish characterized by differences in (1) relative head size, (2) antero-posterior position of dorsal spines, (3) size of posterior process of the pelvis, (4) length of median fins, and (5) length of caudal peduncle. The position of the Cook Inlet Marine Form on the first axis indicates that a relatively large head, posteriorly positioned dorsal spines, shortened pelvis, reduced median fin length and elongated caudal peduncle are derived features of FW sticklebacks. Variation in scores among habitats indicates that these derived features are most strongly divergent in sticklebacks sampled from lakes without native predatory fish (Fig. 3).

PC2 contrasts sticklebacks characterized by differences in (1) orientation of the snout, (2) relative body depth along the length of the body, and (3) length of the caudal peduncle. A shallow body, upturned snout and long caudal peduncle are derived in all habitats except shallow lakes (high relative littoral area) with native predatory fish. Increased body depth is a derived feature of fish inhabiting some of the shallow lakes.

The dominant feature of PC3 is the variation in the length of the ectocoracoid. This variation is not associated with habitat differences, but the position of the Cook Inlet Marine Form, relative to the FW forms, indicates that this element has shortened following the invasion of lakes.

The shape changes from the presumptive marine ancestral shape to the four derived FW shapes are given in Fig. 4. Each deformation is the thin-plate spline of the Cook Inlet Marine Form (the mean of the two Cook Inlet marine group-mean-figures) to the grand-mean figure of the lake-mean-figures of the lakes with the associated habitat variables.

The choice of the marine ancestral form for the computation of the difference vectors has a small effect on their direction. The vector correlation between the difference vectors computed from the Cook Inlet Marine Form and difference vectors computed from individual marine sites were: Kenai, $r = 0.99$; Rabbit, $r = 0.99$; Maine, $r = 0.91$; Oregon, $r = 0.9$; Poland, $r = 0.94$; White Sea, $r = 0.85$.

DISCUSSION

Is the marine form ancestral?

Geographic and temporal variation among modern and fossil sticklebacks suggest that the stickleback complex consists of one or more phenotypically conservative, mainstem marine lineages that frequently colonize freshwater drainages, resulting in numerous, phenotypically diverse, phylogenetically independent and largely ephemeral, derived lineages (Bell, 1987, 1988; Bell & Foster, 1994; McPhail, 1994). One consequence of this hypothesis is that freshwater sticklebacks diverged from a marine ancestor whose phenotype matches that of modern marine stickleback (Bell & Foster, 1994). Deglaciation of Cook Inlet and the founding of its freshwater stickleback populations occurred well after the divergence of Eastern Pacific and Atlantic marine forms (Ortí *et al.*, 1994). This extreme body shape similarity between sticklebacks sampled from Eastern Pacific and Atlantic populations suggests that it is most parsimonious to conclude that there has been little body shape evolution in Eastern Pacific and Atlantic marine populations since their phylogenetic divergence. As a consequence, marine sticklebacks can be considered to present the ancestral phenotype. We used the average of two marine forms sampled from the Cook Inlet region to represent the ancestral phenotype and to estimate the direction of the evolutionary divergence of each freshwater sample. The high vector correlations between mean difference vector taken from the Cook Inlet Marine Form and the mean difference vectors taken from the individual marine samples suggest that any marine ancestor with a body shape within the global

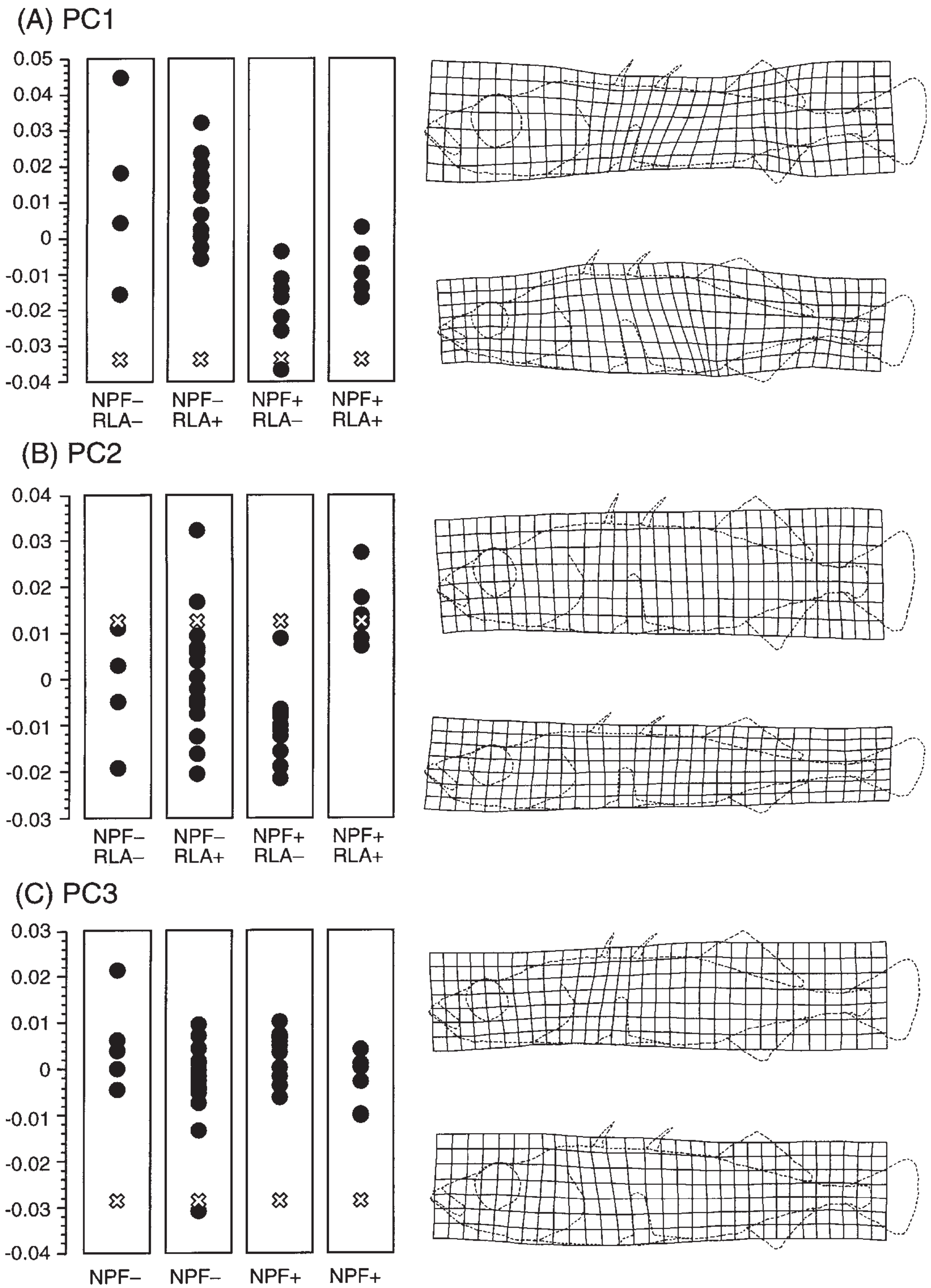

Fig. 3. Shape variation along the first three principal components based on the freshwater data only (PCA_{FW}). The splines indicate the hypothetical extreme shapes on opposite ends of each axis. Each axis represents a continuum of shape variation from one extreme to the other. The ordinations indicate the shape of the derived freshwater group mean figures relative to that of the Cook Inlet Marine Form (the mean of the two Cook Inlet marine group-mean-figures), indicated by the open 'x'. The freshwater fish are subdivided into four groups, based on lake environment: NPF ± indicates the presence (+) or absence (−) of native predatory fishes; RLA ± indicates lakes with high (+) or low (−) relative littoral area. (A) PC1. The axis describes variation in defensive structures and indicates that freshwater sticklebacks have evolved reduced defences relative to the ancestral marine sticklebacks. (B) PC2. The axis describes variation in head shape and body depth and suggests that most freshwater sticklebacks have evolved more shallow body profiles and longer snouts. (C) PC3. The axis describes variation in length of the ectocoracoid and suggests that freshwater populations have reduced ectocoracoid size following colonization. See Discussion for details.

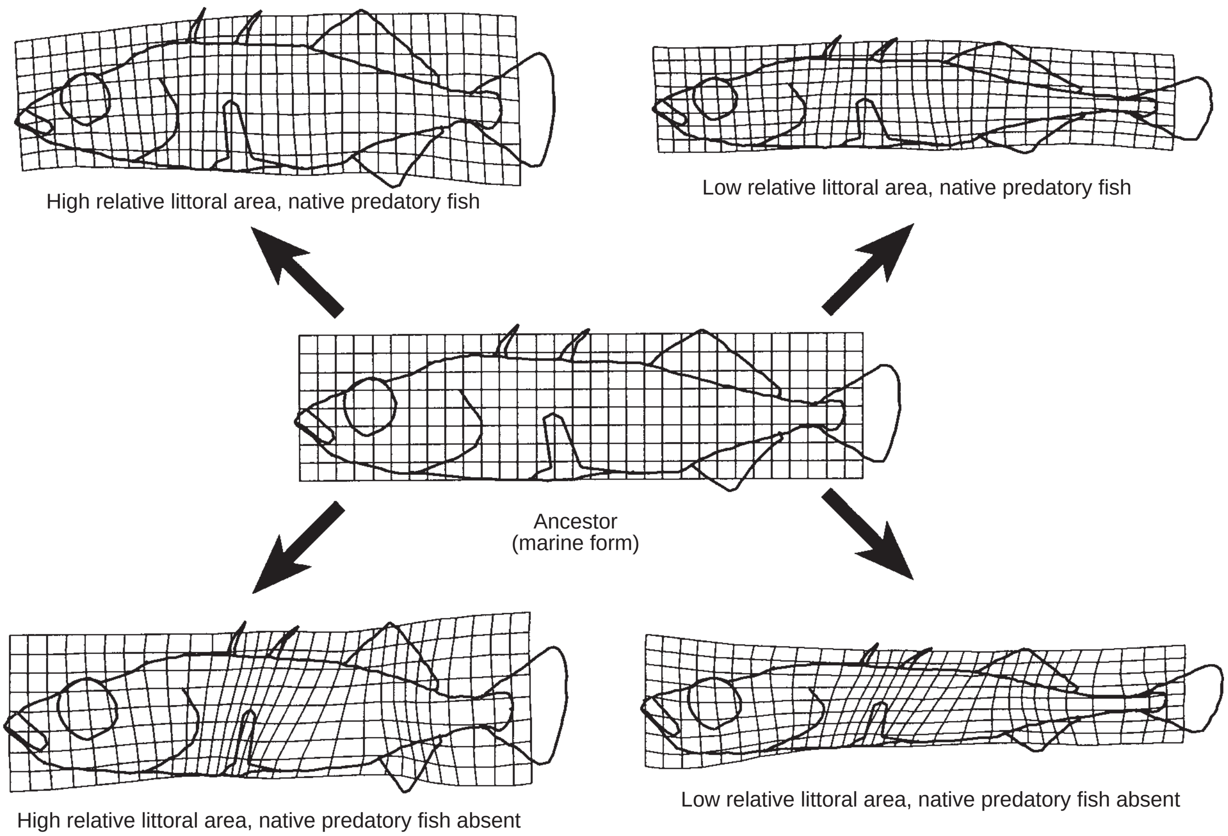

Fig. 4. Summary thin-plate splines of shape change from presumptive ancestral shape (the mean of the two Cook Inlet marine group-mean-figures) to the four derived freshwater phenotypes. Each derived figure is the grand-mean-figure of the lake-mean-figures of the lakes with the associated habitat variables.

range of the marine sites we have sampled would result in similar difference vectors to those estimated using the Cook Inlet Marine Form.

Functional significance of derived phenotypes

Any interpretation of phenotypic variation among derived populations or species without an estimate of the polarity of phenotypic changes is not only incomplete but can suggest misleading evolutionary scenarios. For example, variation in bony armour among freshwater sticklebacks might suggest that some environmental factor has selected for *increased* armour in some populations. Robust bony armour is primitive for freshwater stickleback; its presence in some lake populations is probably best explained by stabilizing selection. To explain the diversity of armour structure present among freshwater populations, we need a deterministic mechanism for not only the maintenance of robust armour but also its characteristic reduction. While there has been intensive research on the function of the armour as a defensive mechanism against predation by piscivorous fish and birds (reviewed in Reimchen, 1994) there has been far less research on factors selecting for reduced armour (but see Reimchen, 1980; Giles, 1983; Bell *et al.*, 1993; Andraso, 1997*a*,*b*).

We suggest that two of the derived traits common to all freshwater populations, the smaller posterior process of the pelvis (the bone immediately anterior to landmark 12) and smaller ectocoracoid (the bone bounded by landmarks 13 and 14), reflect the increased cost of bone growth in fresh water in combination with a decreased functional demand for large bone size. Allocation of energy and mineral resources to bone growth competes with other targets that affect fitness. Teleosts acquire calcium ions by active transport (Dacke, 1979; Koenings, Lipton, & McKay, 1986; Woods & Shuttleworth, 1995; Verbost *et al.*, 1997). Both ion transport and synthesis of transport proteins are energetically expensive (Dacke, 1979; Durham, 1991). Several physiological functions compete with skeletal ossification for calcium (Weiss & Watabe, 1978; Ruben & Bennet, 1987; Takagi & Yamada, 1992), increasing calcium demand. There is good reason to expect that skeletal ossification strongly affects energy and materials budgets, and consequently fitness, of teleost fishes. There is also direct evidence for a trade-off between growth and skeletal ossification (Starck, 1994; Arendt & Wilson, 2000), and domesticated animals selected for rapid growth often have reduced skeletal density (Hedhammer, 1973; Thompson, Jackson, & Baker, 1988; Leterrier & Nys, 1992).

The size of bony elements in freshwater sticklebacks from Cook Inlet probably reflects a compromise between the demands for a structurally adequate skeleton and the high energetic demands of bone tissue. The *calcium limitation hypothesis* (Giles, 1983) suggests that following the colonization of lakes with reduced calcium, there should be net selection for reduced armour structure due to the increased cost of its growth, even if levels of stabilizing selection on the size of the structure due to its function are maintained. With any reduction in the level of the stabilizing selection on an element (spine, lateral plate, pelvis) due to the element's function as an anti-predator mechanism, the magnitude of the net selection for decreased size should be even greater.

Expression of armour varies among freshwater stickleback populations (Hagen & Gilbertson, 1972; Moodie & Reimchen, 1976; Bell, 1984, 1987; Bell *et al.*, 1993; Bell & Ortí, 1994; Reimchen, 1994, 1995), and pelvic reduction is associated with both the absence of predatory fish and lower ionic concentrations (Giles, 1983; Bell *et al.*, 1993; Bourgeois *et al.*, 1994). There is good direct and indirect evidence for selective predation on stickleback armour phenotypes (Hoogland, Morris & Tinbergen, 1957; Hagen & Gilbertson, 1972, 1973; Moodie, 1972; Moodie *et al.*, 1973; Moodie & Reimchen, 1976; Gross, 1978; Reimchen, 1988, 1992, 1994; Banbura, Przybylski, & Frankiewicz, 1989; Bell *et al.*, 1993), in general, and for length of the posterior process (Banbura *et al.*, 1989) in particular. Given the lower concentrations of many ions in fresh water, the smaller posterior process in the lake sticklebacks is consistent with the calcium limitation hypothesis.

The evolutionary reduction of the ectocoracoid is related to the reduced emphasis on swimming in freshwater populations. While most fish use a caudally travelling axial wave to generate propulsive forces, sticklebacks use axial undulation only for rapid accelerations (high curvature turns while manoeuvering and fast-starts during escapes or prey attacks). For many locomotor behaviours, including low-acceleration (not necessarily low velocity) swimming, hovering and nest fanning, sticklebacks rely exclusively on the pectoral fins. The deep adductor muscle, the major muscle active during the power stroke (Drucker & Jensen, 1997; Westneat & Walker, 1997), arises from the ectocoracoid in sticklebacks (pers. obs.). The reduced functional demand of the pectoral musculature could result from either lower activity levels or power requirements. Although activity levels among sticklebacks from different habitats have not been measured, we assume that freshwater sticklebacks have lower levels than their marine ancestors. Juvenile marine sticklebacks make extensive migrations from freshwater streams or coastal estuaries out to sea (Cowen *et al.*, 1991), spend nearly 2 years in the open ocean and return to estuaries or streams in the spring to spawn. Measures of prolonged swimming performance in juvenile marine sticklebacks indicate that they are physiologically able to complete long migrations on their own power (Stevens, 1993), although it is not known if migrations are aided by currents. By contrast, freshwater sticklebacks do not migrate (limnetic populations make short migrations between deeper and shallower waters within a lake) and those from Cook Inlet probably spend much of the winter in a state of torpor. The pectoral fin muscles of freshwater stickleback could have lower size-specific power requirements because of their smaller body mass. The power generated by a muscle is proportional to its cross-sectional area and thus to the square of the individual's length but the inertial mass of an individual is proportional to the cube of its length and, therefore, smaller sticklebacks need disproportionally less pectoral fin muscle to generate the power necessary to accelerate the body. In sticklebacks, the pectoral fins are not only used for small acceleration movements but also aid axial undulation during many high acceleration movements. We suggest that the lower power requirements for the smaller freshwater stickleback resulted in selection for smaller pectoral musculature (not measured) and, as a consequence, smaller ecotocoracoid size.

Three derived traits, pelvic reduction, a more posterior position of the first dorsal spine and reduced median fin length are consistent with the hypothesis that predation pressure is less intense in lakes than in the sea. In contrast to that for pelvic reduction, we offer no causal explanation for the change in spine position or reduction in median fin length. Nevertheless we use the variation in these traits among freshwater stickleback to highlight the importance of polarizing shape variation. Among freshwater populations, the dorsal spines are positioned more anteriorly and the median fins are longer in fish inhabiting lakes with native predatory fish relative to those from lakes without native predatory fish (Walker, 1997). Reimchen (1991) observed that piscivorous fish tend to ingest stickleback headfirst, which suggests that a more anterior position of the spines could increase the incidence of escape during prey handling. Walker (1997) argued that long median fins reflect a locally optimal compromise allowing the ability to rapidly accelerate during an escape response (large fins accelerate a large volume of water) without jeopardizing the efficiency of routine swimming because collapsible median fins have small drag. Without the evidence of the primitive states of this suite of defensive traits, one might 'explain' the variation in spine position and fin length with the hypothesis that there has been directional selection for more anteriorly positioned spines and longer fins in lakes with native predatory fish. But because the ancestral stickleback presumably had anterior spines and long median fins, this hypothesis can explain, at best, the maintenance of the ancestral state. A more thorough research programme on dorsal spines and median fin length would have to investigate the gamut of behaviours in which the spines and fins function in order to identify potential sources of selection for their derived states.

Two body shape characters – snout shape and relative depth along the entire length of the body – evolved in divergent directions. In some shallow lakes, especially those with native predatory fish, body depth increased along its entire length and the snout shortened and rotated more horizontally, while in deep lakes and most shallow lakes without native predatory fish, the opposite changes occurred. The longer snout and more vertically oriented mouth in most lacustrine stickleback is paradoxical. During its marine phase, anadromous stickleback predominantly feed on pelagic zooplankton (Williams & Delbeek, 1989), a behaviour that is typically correlated with relatively long snouts in fish (Liem, 1993). Indeed, within the sympatric pairs of lacustrine stickleback from British Columbia, a relatively long snout is associated with zooplanktivorous diet and increased feeding performance on zooplankton while a relatively short snout is associated with a generally benthic diet and increased feeding performance on zoobenthos (Larson, 1976; Bentzen & McPhail, 1984; Lavin & McPhail, 1986; Ibrahim & Huntingford, 1988; Schluter, 1993).

The shallower body profiles of most lacustrine sticklebacks were the opposite of our expectations based on the biomechanics of uniform velocity cruising (Webb, 1982, 1984). Anadromous sticklebacks, which migrate from the open ocean to estuaries and freshwater rivers to breed (Wootton, 1976), almost certainly swim in the open water for longer periods than freshwater sticklebacks. In general, fish that routinely cruise in open water have shallow, streamlined body shapes in order to reduce hydrodynamic drag (Webb, 1982, 1984). Body depth may also function as a defence against predators. If so, marine sticklebacks may have opposing selection pressures on body size. The shallower body in most freshwater sticklebacks may be a function of reduced predation pressure in the lake environment, relative to the marine environment, a hypothesis that is also supported by the generally gracile armour in freshwater sticklebacks.

The relative contributions of phenotypic plasticity and genetic differentiation to the observed shape differences between anadromous and freshwater populations have not been measured. A number of experiments suggest that trophic and body shape differences among British Columbian populations have a large genetic component (McPhail, 1984, 1992, 1994; Lavin & McPhail, 1993). Day, Pritchard & Schluter (1994) found adaptive phenotypic plasticity for mouth and body shape traits in a pair of sympatric sticklebacks from British Columbia, but the degree of the plasticity could not account for the total difference between the populations.

Significance to studies of morphological evolution

The suites of body shape characters that differ most among sticklebacks from lakes with contrasting environments also have the most influence on the principal directions of variation among populations (the vector correlation between $PC1_{FW}$ and the discriminant axis differentiating sticklebacks from lakes with and without native predatory fish (data from table 6 of Walker, 1997) is 0.98 while the vector correlation between $PC2_{FW}$ and the normalized vector of regression coefficients of each coordinate on relative littoral area (data from table 5 of Walker, 1997) is 0.87. This suggests that biotic and abiotic features of the lakes have a large influence on the among population covariance structure by biasing the locations of phenotypic fitness optima within the multivariate body shape space.

In contrast, Schluter (1996) argued that the pattern of among population covariances among sticklebacks from British Columbia reflects the correlated sampling of phenotypic traits due to the genetic covariance structure within populations (Lande, 1979). This hypothesis assumes that the major axis of interpopulation variation reflects the principal direction of net evolutionary change from marine ancestor to freshwater derivatives. At least for our data on body shape variation, the principal directions of net differentiation and interpopulation variation differ (Fig. 2). The average correlation between the normalized difference vectors and the first eigenvector of the FW covariance matrix is 0.51, indicating that, on average, the estimated direction of net evolutionary divergence and the major axis of interpopulation variation differ by 30.7°. Our results, then, are important for studies inferring genetic constraints on phenotypic evolution based simply on a correlation between within and among population covariance matrices (or their principal components).

In previous work, it was shown that body shape similarities among many freshwater samples are unlikely to have resulted from historical events such as common ancestry or population mixing (Walker, 1997). Given that there are tens of thousands of lakes along the Northwest Pacific coast of North America, the recent radiation of Eastern Pacific freshwater sticklebacks represents a remarkable case of parallel evolution. Our method of using anadromous sticklebacks to represent the ancestral phenotype is not limited to phenotypic means. It would be of great value to repeat this study with measures of genetic and phenotypic covariance structure within anadromous and freshwater populations to explore the evolution of covariances and their influence on the trajectory of phenotypic evolution.

Acknowledgments

We thank S. A. Foster, J. Baker, K. Klebe, and the Alaska Department of Fish and Game for invaluable field assistance. Parts of this research were supported by C. Sexton, a Raney Award from the American Society of Ichthyologists and Herpetologists, a Theodore Roosevelt Memorial Fund award from the American Museum of Natural History, NSF Doctoral Dissertation Improvement Award DEB-9223844, and NSF award DEB-9253718 to S. A. Foster and NSF awards BSR8905758 and EAR9870337 to M. A. Bell. This is contribution 1054 from Ecology and Evolution at the State University of New York at Stony Brook.

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